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Cell Volume Increase in *Escherichia coli* after Shifts to Richer Media

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Synchronous cultures of *Escherichia coli* 15-THU and WP2s, which were selected by velocity sedimentation from exponential-phase cultures growing in an acetate-minimal salts medium, were shifted to richer media at various times during the cell cycle by the addition of glucose or nutrient broth. Cell numbers and mean cell volumes were measured electronically. The duration of the division cycle of the shifted generation was not altered significantly by the addition of either nutrient. Growth rates, measured as rates of cell volume increase, were constant throughout the cycle in unshifted acetate control cultures. When glucose was added, growth rates also remained unchanged during the remainder of the cell cycle and then increased abruptly at or after cell division. When nutrient broth was added, growth rates remained unchanged from periods of 0.2 to 0.4 generations and then increased abruptly to their final values. In all cases, the cell volume increase was linear both before and after the growth rate transition. The strongest support for a linear cell volume increase during the cell cycle of *E. coli* in slowly growing acetate cultures, however, was obtained in unshifted cultures, in complete agreement with earlier observations of cell volumes at much more rapid growth rates. Although cell growth and division are under the control of the synthesizing machinery in the cell responsible for RNA and protein synthesis, the results indicate that growth is also regulated by membrane-associated transport systems.

Regulation of cell division in *Escherichia coli* and *Salmonella typhimurium* was first examined by shifting exponentially growing cultures to richer media (4, 5, 10, 13, 26). These observations indicated that when exponentially growing cultures were shifted to richer growth media (an upshift), the rates of cell division were unchanged for about an hour (4, 5, 10, 13, 26). This rate maintenance of the cell division rate for about 60 min led to the suggestion that the delay reflected a constant time between the initiation of a round of chromosome replication and the cell division following that round of replication (5, 13, 26).

Cell division rates in synchronized cultures did not remain strictly constant; some perturbations occurred (5, 24). Cooper (5) has suggested that these minor changes in cell division rate might reflect correspondingly small alterations in the C and D periods of the cell growth cycle (during and after DNA synthesis) and, therefore, that the concept of rate maintenance could still apply.

Although the effects of shifts between steady states upon cell division rate have been studied extensively, there have been no corresponding studies of cell mass or volume increase. In a related major study, Zaritsky et al. (32) determined cell lengths and diameters in a culture of *E. coli* that was shifted to a richer medium. They compared their results with predicitions of several different growth models and concluded that the best fit was given by a linear surface growth model. The results presented below, however, are the first that have been obtained by measuring cell volumes electronically in cultures that were shifted between steady states.

MATERIALS AND METHODS

Strains and culture conditions. *E. coli* 15-THU (*thy his ura*) and K-12 WP2s (*trp*) were originally obtained from P. C. Hanawalt and E. Witkin, respectively. Cells were cultured overnight at 37°C on a rotary shaker in 30 to 40 ml of an M9 salts solution (27) containing sodium acetate (1%) and the required amino acids (40 μg/ml). For strain 15-THU, thymine was added to 10 μg/ml and uracil was added to 40 μg/ml. Sucrose was added to 2% to prevent osmotic excursions during later selection of cells by velocity sedimentation in sucrose gradients. During the preparation of synchronous cultures, cells were concentrated by filtration, and after selection, the synchronous cohort was returned to the same conditioned medium from which the cells were removed. Nutrient shifts were accomplished by the addition of glucose to 0.1% or nutrient broth to 5%.

Selection of synchronous cultures. When overnight cultures reached a cell concentration of approximately 5×10^7 cells per ml, the cells were concentrated by velocity sedimentation in sucrose gradients, and the smallest cells, at the top of the band, were selected for the synchronous culture, exactly as described earlier (21).

This method of selection has been tested more extensively than any other synchronization technique for possible perturbation of growth (17, 18). Within counting errors, cell number doubling times were the same for the parent culture, the synchronous culture, and a control from which the synchronous cohort was removed. The cell volume doubling period also had the same value as the doubling period of cell numbers, and mean cell volumes in the synchronous cultures were the same as those in the parent exponential-phase cultures from which they were derived. Furthermore, cell buoyant densities were the same in exponential-phase and synchronous cultures (17). These results indicate the absence of any significant perturbation of cell growth by this method.

Counting, sizing, sampling, and resolution. The Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.) multichannel analyzer system described earlier (21) was used to count and to determine the sizes of cells electronically. Because of the small cell volumes in the acetate medium, apertures of 15 to 17 μm in diameter and 30 to 40 μm in thickness were used. The resolution obtained with polysty-

rene latex monodisperse microspheres of 1.099 μm in diameter, expressed as the coefficient of variation of the volume of these particles, was 4.0% (18), corresponding to a resolution of 1.3% in particle diameter. The counter-sizer system was calibrated daily with these particles to determine the absolute values of cell volumes.

Samples (0.5 ml) were taken from growing cultures and diluted into 9.9 ml of HCl (0.1 M) for counting and sizing. Earlier tests established the accuracy and reproducibility of the method (18). Cell counts (for 10-s periods) were taken in quadruplicate or sextuplicate, depending on the culture concentration, with minimal counts of 50 to 60 cells. Cell volume distributions were also obtained, summed, and plotted on a scale of 128 channels and were then used for analysis of the size distributions. Mean cell volumes and standard errors in exponentially increasing cultures were 0.69 ± 0.05 and 0.58 ± 0.03 μm^3 for strains 15-THU and K-12 WP2s, respectively. During most of the cell cycle, cell size distributions were bimodal-containing peaks for mother and daughter cells (15, 17). The data were analyzed by fitting these distributions with normal distributions for each peak (17). As discussed earlier (17), cell growth during the cycle is represented only by the behavior of the undivided mother cells in a synchronous culture, because division produces daughter cells of the following generation. Thus, for an increase in cell volume, it is crucial that only the undivided cell fraction be considered for each generation, as was done in the experiments described below.

Cell growth parameters. Doubling times and mean cell volumes at birth and during the division cycle in synchronous cultures were determined as described earlier (17).

Only the data of the first generation of synchronous growth were used for these determinations, because the data for later generations were convoluted by the accretion of newly born cells during culture division periods. The effect of adding newborn cells is to cause the curves for cell volume increase (CVI) to lag before reaching the actual rate of CVI, as discussed earlier (15). Following this division period, however, the growth curves are again representative of cell growth alone, and rates of growth should agree with those in the first generation. This analysis was supported by the results for the first and second generations of the control culture (see Fig. 1A).

RESULTS

Acetate-glucose shifts. The results of adding glucose to synchronized acetate cultures of *E. coli* 15-THU are shown in Fig. 1A to C for shifts early in the cycle, during midcycle, and late in the cycle, respectively. Figure 1A to C also includes data for the corresponding control synchronous cultures that were not exposed to glucose. The cell count data at the bottom of each panel in Fig. 1 show that the addition of glucose to these cultures had little or no effect on the increase in cell numbers during the first division or on the duration of the first division period. The first division periods were quantitatively compared in eight experiments in which cultures of *E. coli* 15-THU were shifted to richer media at various times from 7 to 66 min after the establishment of synchrony. The average division times ± standard errors were 115 ± 5 and 114 ± 5 min, respectively, in the acetate control and the glucose-shifted cultures. Generation times in subsequent cycles of the shifted culture were always shorter than those for the first (shifted) generation, as was evident from the increased cell counts. Similar results were obtained with the K-12 WP2s strain.

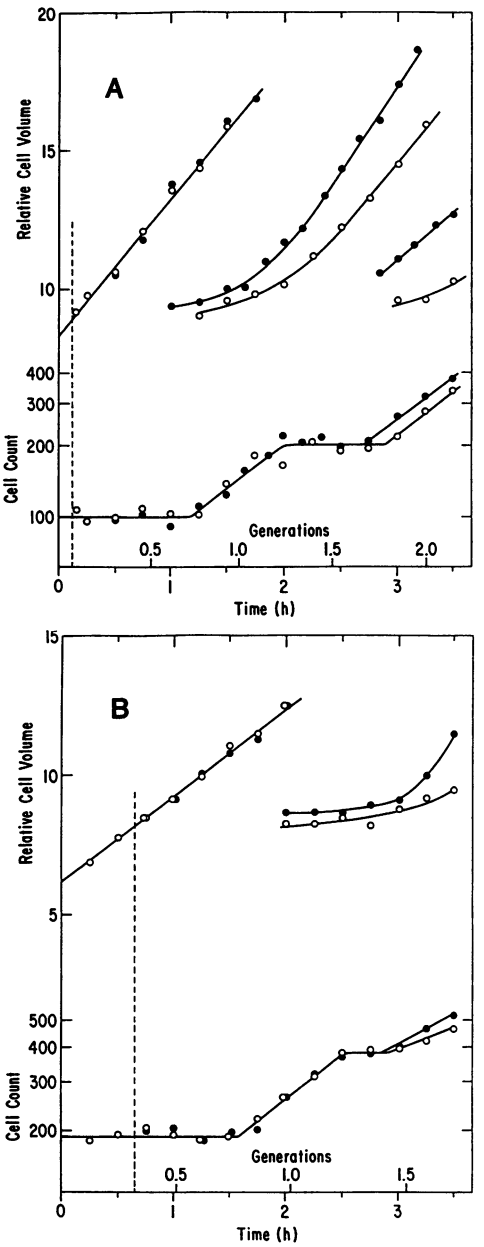
Rates of CVI of undivided (mother) cells during the first cell cycle also appeared to be unaffected by the addition of glucose and increased only after the first division (Fig. 1A to C). This increase was supported by the larger cell volumes after division in the shifted versus those in the unshifted cultures and by the linear increase in cell volume shown in Fig. 1A, as well as in other experiments (data not shown), following the termination of division of the first generation of cells. In order to examine the kinetics of CVI more closely, I plotted the data for the different experiments to a common scale on which the relative cell volume V/V_b was plotted against the cycle age t/T, where t is the chronological age of the culture in generations, T is the generation time in acetate medium, V is the average cell volume observed at time t (for undivided, mother cells), and V_b is the mean cell volume at birth. Methods for calculating V and T for synchronous cultures were described earlier (17, 18). The experimental values are shown for the synchronous controls for both strains (Fig. 2) and for the glucose-shifted cultures of strain 15-THU (Fig. 3). The data in Fig. 2 and 3 were fitted with simple, unconstrained linear or exponential regressions for CVI during the cell cycle, and these regressions were tested for their agreement with the linear and the exponential growth models as discussed below.

On the arithmetic coordinates shown in Fig. 2A, the linear growth model had slope and intercept values of unity. For similar comparisons, the ordinates of the exponential growth model were transformed to $1 + (\ln V/V_b)/\ln 2$ to provide a straight line on semilogarithmic coordinates in Fig. 2B with the same value of unity for the slope and the intercept (17). Regressions were fitted, and values of the slope and the intercept were compared with the model values by the Student t test. The results (Table 1) showed that slope and intercept values were within expectations for the linear growth model ($P > 0.1$ in all cases), whereas observed intercept values deviated significantly from those predicted by the exponential growth model ($P < 0.001$). In addition, examination of the residual values between observed data and theoretical models demonstrated that their distribution was nonrandom for the exponential growth model for strain 15-THU, as well as for the summed data for the two strains. For example, at ages between 0.3 and 0.7 generations, the numbers of positive and negative residual values in Fig. 2A were 20 and 23, respectively, whereas in Fig. 2B the corresponding numbers were 36 and 7. This nonrandom distribution of residual values for the exponential growth model shows that this model is misspecified and therefore invalid (3).

The results in Fig. 3 are of special interest because they support linear growth at unchanged rates of CVI in glucose-shifted cells throughout the remainder of the cycle (Table 1).

Acetate-broth shifts. When cultures of *E. coli* 15-THU were shifted to nutritionally richer media by the addition of nutrient broth, increases in cell numbers during the first synchronous cycle were again essentially the same as those in the control cultures (Fig. 4A to C). Although fluctuations occurred in individual experiments, first-division periods were, on average, no different for shifted and unshifted cultures; the mean times to first division for both upshifted and control cultures of *E. coli* 15-THU and K-12 WP2s were 106 ± 5 min for each strain (nine and six experiments, respectively).

During the period following the addition of broth and expression of the increased growth rate, the results for CVI were indistinguishable for control and broth-supplemented cultures (Fig. 4). Therefore, because the evidence presented



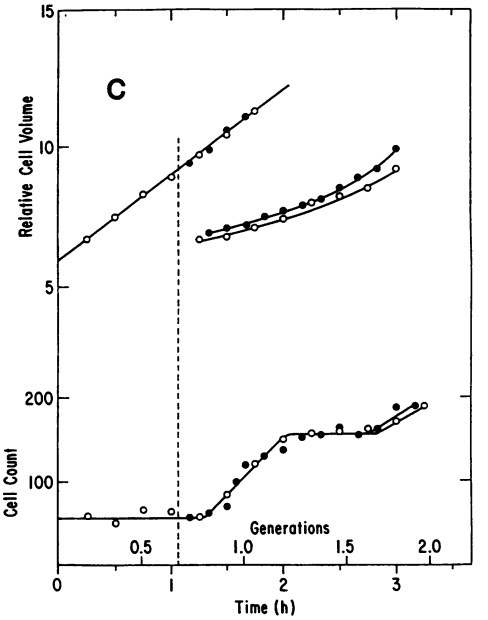


FIG. 1. Shifts to glucose medium for *E. coli* 15-THU. Total cell counts (semilogarithmic coordinates) and relative cell volumes of undivided mother cells (arithmetic coordinates) are shown as a function of the age of the synchronous cultures in terms of chronological time and numbers of elapsed generations. Symbols: ○, unshifted acetate control cultures; ●, cultures shifted up by the addition of glucose to 0.1%. The vertical dashed line indicates the time of the shift. (A, B, and C) Individual experiments in which cultures were upshifted early in the cycle, during midcycle, or late in the cycle, respectively; 10 units of relative cell volume correspond to mean cell volumes of 0.46, 0.70, and 0.71 μm^3, respectively; these factors were chosen for convenience of scale. Viable cell counts per milliliter were 10^4 times the electronic cell count (semilogarithmic scale). Relative cell volumes (arithmetic scale) represent mean cell volumes for successive generations. Overlaps of these curves indicate relative cell volumes of undivided (mother) cells and of divided (daughter) cells that advanced to the next generation.

above (Fig. 1) supports a linear CVI in the control culture, this correspondence between the data of the two kinds of experiments also supports a linear CVI prior to the growth rate shift in the broth-supplemented cultures. Admittedly, the numbers of comparative datum points in this region are relatively few, with one, two, and six datum points in Fig. 4A to C, respectively; but this agreement was observed in each of the 15 experiments that were undertaken.

In contrast to the results for shifts to glucose-containing media, however, rates of CVI usually increased sooner after the upshift and to higher values (Fig. 4A to C). If cultures were shifted by midcycle, the faster growth rate was established during the same generation and was maintained in successive generations, as was evident from the parallel slopes for CVI in successive generations (Fig. 4A). As explained earlier (15), the initial curvature in CVI just after the onset of cell division is an artifact arising from the incomplete division of cells in the culture. The true shape of the curve for CVI became evident only after all cells of any particular generation divided, and in Fig. 4A it was again seen to be linear.

The duration of the delay period before the onset of the faster growth rate in the shifted cultures was dependent on the age of the synchronous culture at the time of the nutrient shift (Fig. 5). The delay in the growth rate shift, approximately 0.4 generations when cultures were shifted early in the cell cycle, decreased as shifts were performed later and then increased again when the cultures were shifted so late in the cycle that the growth rate was altered in the following cycle. Growth rates were shifted up by a factor of 3.5.

DISCUSSION

As described above, rates of cell division and CVI in undivided cells of *E. coli* 15-THU or K-12 WP2s were unchanged for extended periods after shifts to nutritionally richer media. The durations of the division cycles remained unchanged both in glucose-shifted and in broth shifted cultures, and cell division rates increased only during the following cycle. Delay periods before growth rates shifted, however, were dependent on the medium after the shift. In glucose-shifted cultures, growth rates remained constant

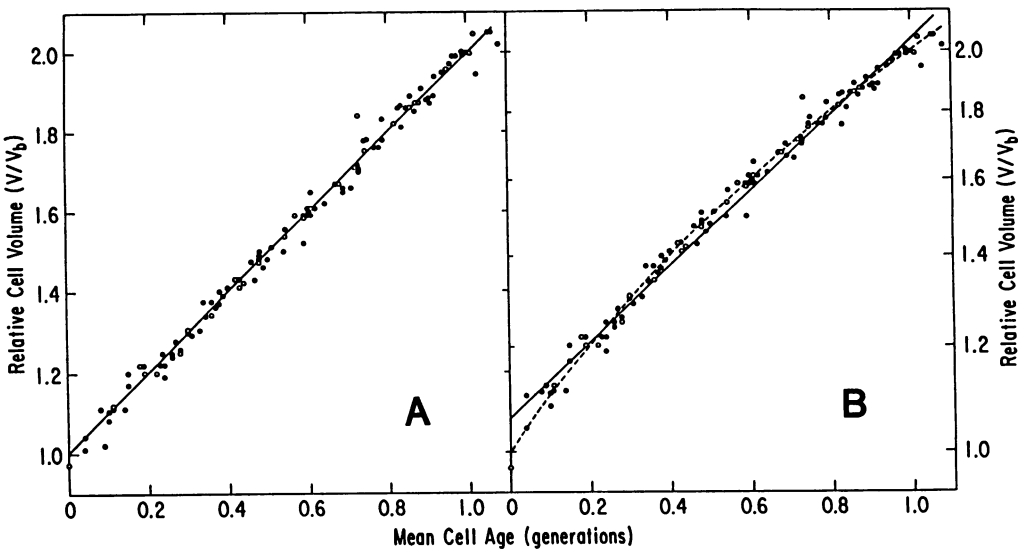


FIG. 2. Relative mean cell volumes during the cell cycle in *E. coli* 15-THU and K-12 WP2s. Observed cell volumes (in units of the mean cell volume at birth) are plotted as a function of culture age for strains 15-THU (●) and K-12 WP2s (○) on arithmetic coordinates (A) and semilogarithmic coordinates (B) to compare the validity of the linear and exponential growth models. The straight lines are unconstrained linear regressions fitted to the data. The dashed line in panel B shows the linear regression of panel A as it appears on semilogarithmic coordinates, thus allowing a direct visual comparison between the linear and exponential growth models. For both models, the straight-line regressions must pass through the value of 1 relative cell volume at the age of 0 generations and through the value of 2 relative cell volumes at the age of 1 generation. These graphs, and those in Fig. 3 and 5, represent the data from all of the experiments.

throughout the remainder of the shift cycle and then increased abruptly with or immediately after cell division. In broth-shifted cultures, growth rates increased only after a sharply defined delay of 0.2 to 0.4 generations. The sharpness of the growth rate steps after the addition of broth is particularly evident in Fig. 4A and B.

The results for constant peroids of cell division are in agreement with most earlier findings, although they fail to support the minor perturbations observed by Loeb et al. (24) in experiments with synchronized acetate cultures of *E. coli* B/r. It is possible that those perturbations were due to a strain-dependent effect, although that explanation is not supported by my observation of constancy in two unrelated strains, *E. coli* 15-THU and *E. coli* K-12 WP2s. Other evidence supports a different explanation, which is that the establishment of synchronized cultures by the membrane elution technique perturbed cell growth. First, Boyd and Holland (1) found that when cells of *E. coli* B/r became attached to membranes, these cells were induced to synthesize protein D and related groups of outer membrane proteins, thereby altering the chemical composition of membrane-synchronized cells from the steady-state value. Second, although the buoyant densities of *E. coli* B/r cells were independent of cell age and growth rate in steady-state cultures, the densities were significantly increased after synchronization by membrane elution (19, 20). Third, as observed by Olijhoek and Nanninga (cited in reference 28), rates of cell length and volume increased in cultures that were synchronized by membrane elution were found to undergo periodic changes during the division cycle. Taken together, these results provide strong evidence for the perturbation of cell growth by the membrane elution technique.

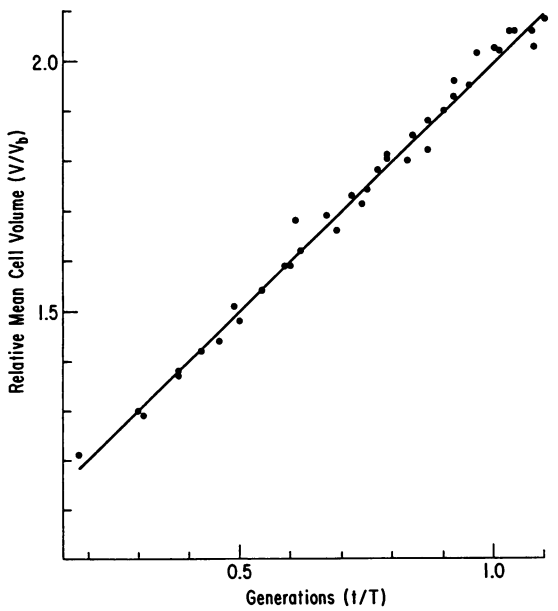


FIG. 3. Relative mean cell volumes during the cell cycle in *E. coli* 15-THU after the addition of glucose at 0 generations. The straight line is the linear regression to the data.

Adherence of *S. typhimurium* to mouse epithelial cells also requires the synthesis of new bacterial proteins that are apparently induced coordinately by structures on the surfaces of epithelial cells (8). Such induction processes could help to account for both the relatively small fractions of cells that adhere to membrane filters and the initial time periods before the culture is regarded as synchronized (9). Other than the examination for synchronized doubling at the time of cell division, tests for the absence of perturbation usually have not been carried out. In contrast, I showed earlier (18) that in synchronous cultures obtained by velocity sedimentation in sucrose gradients, values for both cell division

TABLE 1. Significance tests of regression parameters for cell growth

Growth model	Strain	No.[a]	Slope ± SE	P_1[b]	Intercept ± SE	P_2[c]
Linear	K-12 WP2s	24	1.009 ± 0.023	>0.50	0.997 ± 0.013	>0.50
	15-THU	88	1.013 ± 0.010	>0.20	0.991 ± 0.006	>0.10
	15-THU, s[d]	33	1.012 ± 0.019	>0.50	0.995 ± 0.014	>0.50
Exponential[e]	K-12 WP2s	24	0.991 ± 0.018	>0.50	1.068 ± 0.016	<0.001
	15-THU	88	0.981 ± 0.013	>0.10	1.065 ± 0.008	<0.001
	15-THU, s[d]	33	0.917 ± 0.023	<0.002	1.111 ± 0.016	<0.001

[a] Number of observations.
[b] Probability that the slope is unity.
[c] Probability that the intercept is unity.
[d] Data were obtained after nutritional shift.
[e] Value for cell volume V transformed to $1 + (\ln V/V_b)/\ln 2$.

periods and mean cell volumes at the time of cell division agree with those for the exponentially growing cultures from which the synchronous cultures were selected. These results were confirmed in the experiments described here, which provide support for the absence of any significant perturbation of cell growth in cultures synchronized by the technique used for my studies.

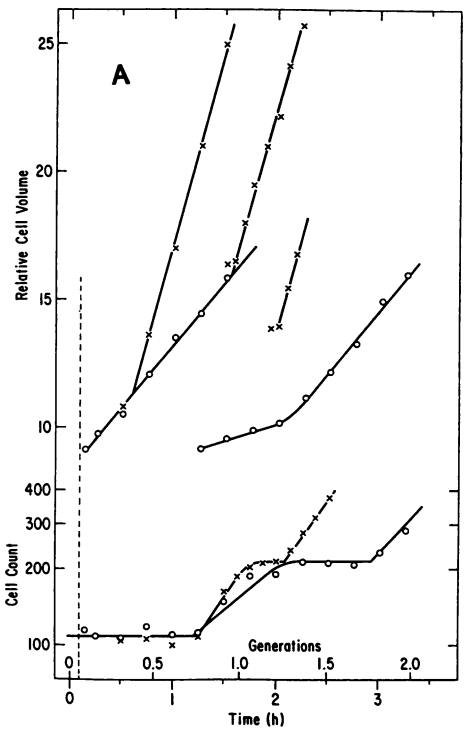
The results for CVI in Fig. 2 provide strong evidence for linear cell growth during the cell cycle. Earlier, it was shown (17) that cell volumes increase linearly in glucose cultures of *E. coli* B/r at doubling times of 25 and 40 min. The results in Fig. 2 extend that conclusion to the two strains 15-THU and K-12 WP2s at a much longer doubling time (106 min). Furthermore, whereas the data in Fig. 2 agree well with a linear CVI, they also make exponential models of growth extremely unlikely (Table 1). The strongest evidence against an exponential CVI is the nonrandomness of the distribution of residual values, which invalidates this model. The counterclaim by Cooper (6) against similar earlier results for *E. coli* B/r was shown to be without basis (23). Furthermore, Cooper (6) did not measure leucine uptake, as also claimed, because soluble leucine pools that were formed at the time of labeling were long dissipated before his measurements could be performed, and unknown fractions of these pools were incorporated into protein.

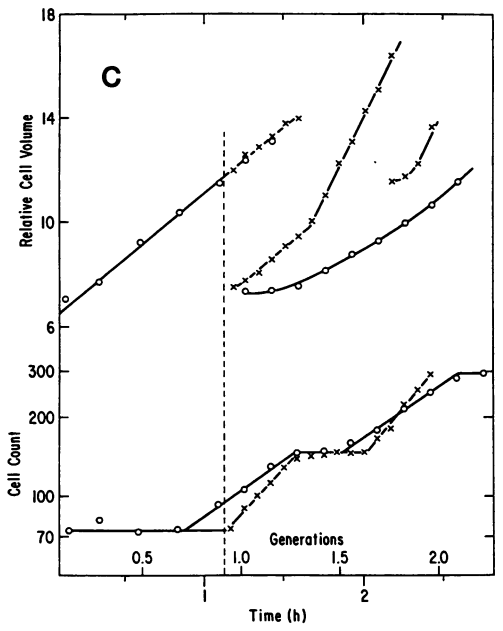
My observation that the data for relative cell volume in shifted cells of *E. coli* 15-THU are fitted well by linear regression (Fig. 3 and Table 1) also provides strong evidence for the absence of a shift in the rate of CVI during the generation when glucose was added. Again, the results in Table 1 also make exponential growth after the shift extremely unlikely.

While growth rates did not change until cell division in glucose-shifted cultures, growth rates in broth-shifted cultures increased abruptly after 20 to 40 min. These different results provide the first evidence that the growth kinetics of the shift depend on the nutrient medium. Furthermore, the response in nutrient broth argues against a popular model of cell growth regulation, which specifies that growth rate doubling coincides with the duplication of a particular gene whose product limits growth. Many other kinds of models for cell growth have also been proposed (see, for example, Zaritsky et al. [32]). In tests of a number of these models, Zaritsky et al. (32) found that their nutrient shift experiments appeared to support the gene-controlled growth rate model mentioned above, with a linear increase in cell surface area and a gradual increase in growth rate after the nutrient shift. Their analysis failed to support the model of Pierucci (30) that new cell envelope growth is activated with the initiation of chromosome replication and continues until completion of that round of synthesis. They did not, however, examine the model that describes my data for the broth-shifted cultures given above, namely, that growth rates were constant and abruptly shifted to new final values at times that were dependent on the age of the cell at the time of nutrient addition. It is apparent that the method I used here for the measurement of CVI has the major advantage of greatly improved resolution over the electron micrographic approach (32), providing smoother curves for cell volume kinetics.

Kepes and Kepes (12) have observed that CVI, measured electronically, is linear in quasi-steady-state cultures of *E. coli*. These cultures, which were synchronized by repeated, twofold dilutions of P_i for many generations, can remain synchronized for six generations or more. Ultimately, in the absence of further synchronization, they must revert to the exponential growth phase. These results do show, however, that CVI can be linear under other growth conditions.

The extensive delays observed between nutrient upshifts and increased rates of cell division and CVI provide evidence that the regulation of growth is a complex phenomenon involving more than the RNA- and protein-synthesizing systems. Indeed, if only those systems were involved, one might expect to find more gradual changes in CVI after the nutritional upshifts as rates of synthesis of envelope components increased. Initially, the sharper changes in growth rate suggested that glucose might have failed to enter the cells in synchronized cultures prior to cell division or that rates of RNA synthesis were not increased in these cultures. Several experiments were performed to test those possibilities. First, a small amount (0.1 ml) of [^{14}C]glucose was added to an exponentially growing acetate culture of *E. coli* 15-THU. Uptake of the label began immediately, increased to about 50% of its final plateau value within 5 min, reached the plateau value at about 30 min, and maintained this plateau for at least 3 h (data not shown). Second, the net uptake rate of [^{14}C]uracil maintained at a constant concentration was observed to be immediately increased by the nutrient shift, in the same manner as that described by Maaløe and Kjeldgaard (26). Third, under the same conditions, the net uptake rate of labeled sodium acetate was decreased by the shift. These results support the conclusion that the cells accumulate glucose during the shift and that RNA synthesis is increased, but that these processes do not affect CVI before cell division. Because cell volume is theoretically determined by the space enclosed by the outer cell membrane (18), which was confirmed experimentally (22), these experimental results indicate an absence of effect of the glucose upshifts on the rate of cell envelope expansion before cell division.





One may ask why the delays for glucose and broth upshifts were so different and whether this difference was due to a component, for example, in the nutrient broth. Some light is thrown on these questions by the recent findings of R. D'Ari (personal communication) that the removal of acetate from the rich glucose medium provided much shorter delays after shifts of exponentially growing cultures of *E. coli* K-12 to richer media. He suggested that this shortening may be related to the excretion of acetate by *E. coli* during growth on glucose. In any case, his results support the view, established by the results of my own experiments, that growth delays are quite variable and are highly dependent on

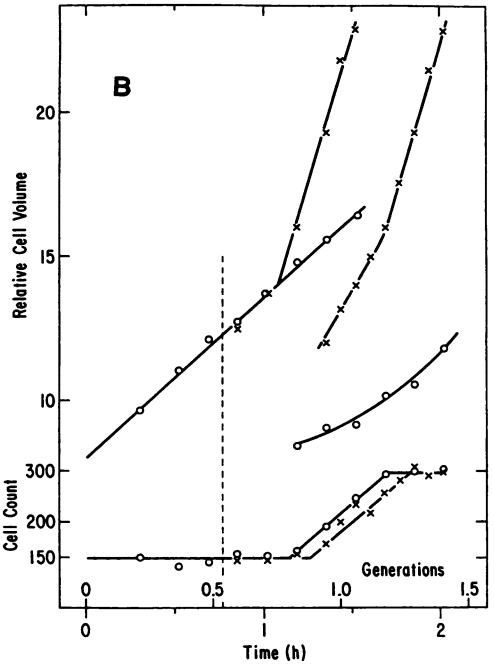


FIG. 4. Shifts to broth for *E. coli* 15-THU. Symbols: ○, unshifted acetate control cultures; ×, cultures shifted up by the addition of nutrient broth to 5%. (A to C) Ten units of relative cell volume correspond to mean cell volumes of 0.71, 0.80, and 0.66 μm^3, respectively. Other symbols are as described in the legend to Fig. 1.

the nature of the medium in which the cells are growing after the shift. This viewpoint does not allow growth models that depend on duplication of a particular gene whose product limits growth.

The extensive delays between nutrient upshifts and increased rates of cell division and CVI also provide evidence for the participation of transport systems in the regulation of cell growth. According to the model of Maaløe and colleagues (11, 25), regulation of growth at the whole-cell level is based on both passive and active regulation. Passive

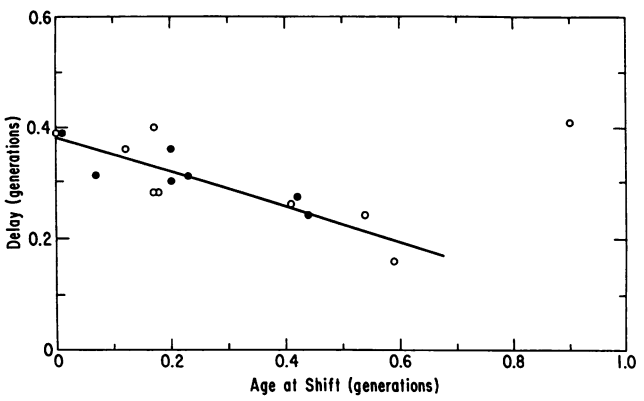


FIG. 5. Delay periods between the addition of nutrient broth and the onset of increased growth rates. Delay periods are shown as a function of age of the synchronous culture. The high value at about 0.9 generations reflects the fact that the shift in growth rate occurred in the next cell cycle after the nutrient shift. Symbols: ○, *E. coli* 15-THU; ●, *E. coli* K-12 WP2s.

regulation is provided mainly by the protein-synthesizing system. Active control has been ascribed to the adjustment of synthesis rates of ribosomal protein and all the proteins of the protein-synthesizing system, although the detailed relationships are unknown. I suggest that the delay in CVI contributes to active regulation by maintaining earlier rates of transport of materials into the cells for extended periods of time during which other mechanisms of active control of the protein-synthesizing system can be accommodated.

Earlier, I proposed (16) that rates of increase in cell volume and mass in *E. coli* are limited by and proportional to the net rate of transport of materials into the cell, which, in turn, is proportional to the number of functional transport sites on the cell surface. Then, cell growth rate constancy during the steady-state cycle requires that the numbers of these sites remain constant during the cycle and be doubled at or near the time of cell division, so that daughter cells again have, on average, the same numbers of sites per cell. The abrupt doubling of some cell components predicted by this model was observed later for a number of envelope proteins (2, 29), as well as for binding proteins for galactose and maltose (7, 31). This model also predicted that soluble pools of precursors for RNA and protein synthesis would increase to a midcycle maximum and then decrease again, in contrast to the predictions of exponential or bilinear growth models. Soluble pools for uracil, histidine, and methionine were measured and observed to follow the kinetics predicted by linear growth (23).

This model relating CVI with transport sites is also consistent with the observed distribution of generation times in *E. coli*. If one assumes a large number of cell transport sites, one would expect a normal distribution of the numbers of these sites per cell. Consequently, the rate at which cell mass increases should also be normally distributed, and the cell generation times should be distributed as the inverse of the mass doubling rate. The experimental evidence strongly supports this distribution (14).

The maintenance of a constant growth rate during the delay period after a nutrient upshift requires that the total number of functional transport sites does not increase immediately after the upshift, but only after the period of delay. According to this scheme, after an upshift with glucose, the number of sites remains constant until cell division. After an upshift with broth, the number of sites would remain constant for 0.2 to 0.4 generations and would then increase abruptly. Presumably, the length of the delay depends on the time required for the appropriate reorientation of the protein-synthesizing system, which might, in turn, provide the signal for an increase in transport proteins. Whatever the detailed mechanism, however, the transport of materials into the cell appears to involve a system of major importance in the regulation of cell growth.

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